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HUMAN HEALTH ASSESSMENT OF ALCOHOL-TO-JET (ATJ) SYNTHETIC KEROSENES

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14. ABSTRACT
This project summarizes the toxicological investigation of two types of alcohol-to-jet (ATJ) fuels intended for use in a 50:50 blend with the petroleum-derived jet fuel JP-8. The ATJ synthetic paraffinic kerosene (SPK) fuels evaluated were produced by Gevo (Englewood CO). The ATJ synthetic kerosene with aromatics (SKA) fuels were produced by Swedish Biofuels AB (Stockholm). The objective of this report is to summarize available toxicological data for ATJ fuels, to compare those fuels among themselves, with petroleum-derived JP-8, and with other alternative fuels for which toxicity data exist, and to recommend an occupational exposure limit (OEL) to protect military personnel exposed to these fuels. All toxicity tests were performed with the fuels following the addition of a standard additive package required for JP-8. Both types of ATJ fuels were evaluated for dermal irritation potential in rabbits and mutagenicity potential using both mammalian and bacterial methods. Ninety-day studies were performed with Gevo (bio) ATJ SPK and Swedish Biofuel SB-8. In two separate studies, male and female Fisher 344 rats were exposed to target concentrations of 0, 200, 700, and 2000 mg/m3 of fuel, 6 hours per day, 5 days a week for 69 or 70 exposure days. Neurobehavioral assays and reproductive health evaluations were included in the study designs. ATJ fuels were found to have similar toxicity or somewhat lower toxicity than petroleum-derived JP-8. Handling of ATJ fuels alone or in a blend with petroleum-derived JP-8 is unlikely to increase human health risks in the military workplace.

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PREFACE

Support for the research described herein was provided through the former Alternative Fuels Certification Office (AFLCMC/WNN) and the Air Force Research Laboratory, Fuels and Energy Branch (AFRL/RQTF). Partial funding was provided from the Office of the Secretary of Defense (OSD) Coalition Warfare Program (CWP). Portions of the research were performed in support of an international agreement between AFRL/RQTF and Sweden. Partial funding was also provided by the Aerospace Toxicology Program in the Air Force Research Laboratory, 711th Human Performance Wing, Airman Systems Directorate, Bioeffects Division, Molecular Bioeffects Branch (711 HPW/RHDJ).

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1.0 SUMMARY

Alcohol-to-jet (ATJ) alternative fuels are produced by several dehydration and oligomerization processes using ethanol or butanol feed stocks. Non-renewable (petroleum-derived) or renewable hydrocarbon sources (sugars or lignocellulosic biomass) can be utilized; the resulting fuel composition is flexible based on production requirements. This report summarizes the toxicological investigation of two very different types of ATJ fuels intended for use by the U.S. Air Force in a 50:50 blend with the conventional petroleum-derived jet propulsion fuel JP-8. The ATJ synthetic paraffinic kerosene (SPK) fuels evaluated in this report were produced by Gevo (Englewood CO). Labelled Gevo ATJ SPK (non-bio) and Gevo ATJ SPK (bio), these fuels were produced from isobutanol feedstocks originating from petrochemical (non-bio) or renewable (bio) sources such as grains (corn, wheat, sorghum, barley, etc.) or sugarcane and contain more than 99 percent aliphatic compounds. The ATJ synthetic kerosene with aromatics (SKA) fuels evaluated were produced by Swedish Biofuels AB (SB, Stockholm) as part of an international agreement between Sweden and the Air Force Research Laboratory Fuels and Energy Branch (AFRL/RQTF) at Wright-Patterson Air Force Base (AFB) OH. These fuels were produced from lignocellulosic sources (grain crops or wood) and contain a minimum of 70 percent branched paraffinic and 15 percent aromatic compounds. The original fuel, SB ATJ SKA (old), was produced in 2010 and contained solely trimethylbenzenes in the aromatic portion of the fuel. The newer fuel, SB ATJ SKA (new) or simply "SB-8", was formulated in 2012 to be more similar to petroleum-derived JP-8; SB-8 contains multiple alkylbenzene components that comprise the 15 percent aromatic fraction of this fuel.

The objective of this report is to summarize available toxicological data for ATJ fuels; to compare the fuels described above with each other, with petroleum-derived JP-8, and with other alternative fuels for which toxicity data exist; and to recommend an occupational exposure limit (OEL) to protect Department of Defense (DoD) personnel exposed to these fuels. All toxicity tests were performed with the fuels following the addition of a standard additive package required for JP-8. Both types of ATJ fuels were evaluated for dermal irritation potential in rabbits and mutagenicity/genotoxicity using mammalian and bacterial methods. Ninety-day studies were performed with both Gevo (bio) and SB-8. In two separate studies, male and female Fisher 344 rats were exposed to target concentrations of 0, 200, 700, and 2000 mg/m^3 of fuel, 6 hours per day, 5 days a week for 69 or 70 exposure days. Neurobehavioral assays and reproductive health evaluations were included in the study designs. ATJ fuels, specifically Gevo (bio) and SB-8, were found to have similar toxicity or somewhat lower toxicity than the petroleum-derived JP-8 in use by DoD today. Therefore, handling of ATJ fuels alone, or in a blend with petroleum-derived JP-8, is unlikely to increase human health risks in the military workplace. Therefore, the occupational exposure limit levels for both the Gevo (bio) ATJ SPK and the SB ATJ SKA (new) "SB-8" fuel is recommended to remain the same as for JP-8: 200 mg/m^3 (vapor) and 5 mg/m^3 (aerosol).

2.0 INTRODUCTION

Alcohol-to-jet (ATJ) alternative fuels are produced by several dehydration and oligomerization processes using ethanol or butanol feed stocks. Renewable hydrocarbon sources such as fermented sugars and lignocellulosic biomass can be used (Satyarthi *et al.*, 2013) and the resulting fuels composition can be tailored based on production requirements. This report summarizes the toxicological investigation of two very different types of ATJ fuels intended for use by the U.S. Air Force in a 50:50 blend with the petroleum-derived jet propulsion fuel JP-8.

All fuels discussed in this report are designated using POSF log book numbers provided by AFRL/RQTF (formerly known as the Air Force Wright Aeronautical Laboratories, AFWAL/POSF). Composition analyses were performed with the neat fuels. All toxicity tests were performed with the fuel following the addition of a standard additive package required for JP-8; the additive package comprises less than 0.15 percent by weight. Alternative fuels have two POSF numbers, one designating the fuel alone and one for the fuel plus additives. These numbers are cataloged in Appendix A.

Use of commercial names and products does not constitute endorsement of these products by the U.S. Air Force, U.S. Navy, or U.S. Army.

2.1 ATJ Fuel Composition

ATJ fuels can be either synthetic paraffinic kerosene (SPK) fuels, containing no aromatic components, or synthetic kerosene with aromatics (SKA) fuels, which contain alkanes similar to those found in petroleum derived JP-8. ATJ SPK fuels are produced by the oligomerization of short-chained alcohol feedstocks into 12 to 16 carbon chain alkanes (Milbrandt *et al.*, 2013). The ATJ SPKs evaluated in this report were produced by Gevo (Englewood CO). Labelled Gevo ATJ SPK (non-bio) and Gevo ATJ SPK (bio), these fuels were produced from isobutanol feedstocks originating from petrochemical (non-bio) or renewable (bio) sources such as grains (corn, wheat, sorghum, barley, etc.) and sugarcane (Gevo, 2011).

The ATJ SKA fuels evaluated herein were produced by Swedish Biofuels AB (Stockholm) as part of an international agreement between Sweden and the Air Force Research Laboratory Fuels and Energy Branch (AFRL/RQTF) at Wright-Patterson Air Force Base (WPAFB) OH. These fuels were produced from lignocellulosic sources (grain crops or wood) and contain a minimum of 70 percent branched paraffin and 15 percent aromatic compounds (SB, 2015). The original fuel, SB ATJ SKA (old), was produced in 2010 and contained solely trimethylbenzenes in the aromatic portion of the fuel. The newer fuel, SB ATJ SKA (new) or simply “SB-8”, was formulated in 2012 to be more similar to petroleum-derived JP-8; SB-8 contains multiple alkylbenzene components that comprise the 15 percent aromatic fraction of this fuel.

A composition comparison of the Gevo fuels, the SB fuels, and a representative petroleum-derived JP-8 can be found in Appendix B. All fuels were analyzed by comprehensive two-dimensional gas chromatography (GC x GC); more detailed analyses of the fuel constituents are found in Sterner *et al.* (2014a). The GC x GC method is detailed in Striebich *et al.* (2014).

2.2 Objective

The objective of this report is to summarize available toxicological data for ATJ fuels, to compare those fuels with each other, with petroleum-derived JP-8, and with other alternative fuels for which toxicity data exist, and to recommend an occupational exposure limit (OEL) to protect DoD personnel exposed to these fuels.

3.0 TOXICITY TESTING OF GEVO ATJ FUELS

Toxicity testing comparison tables for Gevo ATJ fuels are found in Appendices C through G of this document. These tables serve not only to summarize the data found here in Section 3, but also aid in the comparisons laid out in Sections 4 and 5.

3.1 Dermal Irritation

Two SPK fuels with additives, Gevo ATJ SPK (non-bio) (POSF 7699) and Gevo ATJ SPK (bio) (POSF 10021), were tested to determine dermal irritation potential of the neat fuel. Petroleum-derived JP-8 with additives (POSF 4658+) was included in the study for comparison. Following standardized test guidelines (Draize, 1965; OECD, 2002; U.S. EPA, 1998a), New Zealand white rabbits were exposed to each fuel for four hours with exposure sites either semi-occluded or occluded. Dermal irritation was evaluated at 30 and 60 minutes post-exposure, at 24, 48, and 72 hours, and on study days 4, 7, and 14. Semi-occluded exposure to all three jet fuels resulted in scores of "slightly irritating." Occlusion of the test sites raised the dermal irritation index to moderately irritating for petroleum derived JP-8 and Gevo (bio), while Gevo (non-bio) remained slightly irritating. Neither Gevo ATJ SPK fuel is anticipated to increase dermal irritation when handling the fuel in an occupational setting, either alone or in a 50:50 blend with petroleum-derived JP-8 (Sterner *et al.*, 2014a). These data are summarized in Appendix C.

3.2 Inhalation Toxicity

A different, chemically identical batch of Gevo ATJ SPK (bio) with additives (POSF 10263) was used in a 90-day inhalation study with male and female Fischer 344 rats (10 rats per sex per concentration). Rats were exposed to 0, 200, 700, or 2000 mg/m^3 fuel in an aerosol/vapor mixture for 6 hours per day, five days per week for 70 exposure days, per standard test guidelines (OECD, 2009; U.S. EPA, 1998b). Average exposure concentrations, measured by Fourier transform infrared (FTIR) spectrophotometry (aerosol + vapor), were found to be -0.13 ± 0.15, 200.6 ± 2.3, 705.6 ± 13.7 and 2001.8 ± 29.5 mg/m^3 (± standard deviation (SD)) in the control, low, medium, and high concentration groups, respectively. Average aerosol concentrations were measured using gravimetric filters and were 1.22 ± 1.36, 1.74 ± 1.4, 3.54 ± 0.76, and 46.6 ± 5.5 mg/m^3 (± SD), for the control, low, medium, and high concentration exposure chambers, respectively; aerosols represented 0.9 (low), 0.5 (medium) and 2.3 (high) percent of the total average jet fuel concentration. The aerosol concentrations measured in the

control and the low group chambers are attributed to background particulates associated with the presence of animals, such as dander, that are not related to the fuel. Body weights and food consumption did not differ between exposure groups throughout the study (Sterner *et al.*, 2015a).

Following the final exposure, rats were euthanized in accordance with current American Veterinary Medical Association (AVMA) guidelines (2013). Total white blood cells (WBCs) in exposed male rats increased with increasing concentration of Gevo (bio) fuel. Only the 2000 mg/m^3 exposure group WBC count was statistically higher than the control value; the increase was not attributable to a significant dose-response increase in any one WBC subtype. Analysis of the WBC count data indicates that, due to high variability among the control rats (6.88 ± 1.06 cells/μL), it is unclear if the WBC count in the 2000 mg/m^3 exposure group males (7.83 ± 0.73 cells/μL) is biologically significant (Sterner *et al.*, 2015a) as it falls within a single standard deviation and normal range for WBC counts (*i.e.*, 2.06 to 9.61 cells/μL; Charles River Laboratories International, Inc., 2009). No further exposure-related differences were observed in hematology or clinical pathology endpoints. Male rat kidneys were analyzed for α_{2u}-globulin, a male rat-specific protein that may accumulate to form hyaline droplets in renal tubular cells. Hyaline droplet formation is also linked to hydrocarbon exposure. Predictably, α_{2u}-globulin levels increased significantly with increased Gevo fuel exposure (Sterner *et al.*, 2015a).

Histopathological changes were limited to kidneys, nasal cavities and lungs. Mild to moderate irritation-related lesions in the nasal respiratory epithelium occurred in both males and females among the 2000 mg/m^3 exposure group. Minimal severity lesions in the olfactory epithelium of the nasal cavity occurred with low frequency among the 700 mg/m^3 (aerosol + vapor) exposed rats; these lesions did not appear to exceed background (control) incidence. Mild to moderate intensity lesions were found with frequency only among the high concentration group. Minimal to mild goblet cell hyperplasia occurred only in the high exposure group. Female rats exhibited more nasal lesions than male rats across both types of epithelium. Inflammatory infiltrates were observed in the lungs due to focal deficits in clearance; infiltrates were increased in number and severity in the high exposure group likely due to the irritation of fuel exposure. Early stage chronic progressive glomerulonephropathy (CPG) was observed in all rats and is an age-related disease common in F344 rats; however, the severity of findings increased with high levels of fuel exposure in male rats due to hydrocarbon nephropathy (Sterner *et al.*, 2015a). This protein-overload disease is generally considered to be male rat specific and not pertinent to human health (Borghoff *et al.*, 1990; Hard *et al.*, 1993). Overall, the 90-day study with Gevo (bio) resulted in a high incidence of mild to moderate nasal lesions in rats exposed to 2000 mg/m^3 (Sterner *et al.*, 2015a). These effects are tabulated in Appendix D.

3.3 Reproductive Health

As an indicator of reproductive health, vaginal cytology was assessed during week 9 of exposure. Sperm count, motility and morphology were evaluated immediately after the full 70 days of exposure. No abnormalities were found for males or females across the exposure groups (Sterner *et al.*, 2015a). Reproductive toxicity results are tabulated in Appendix E.

3.4 Neurobehavioral Effects

During weeks 13 and 14 of the study, motor activity assays and the functional observational battery (FOB) tests were performed following exposure to assess potential neurobehavioral effects, per U.S. EPA guidelines (1998c). No significant differences were found between exposure groups among the male rats for motor activity or FOB. There were no significant differences in motor activity for female rats, with the exception of total activity time, where the high exposure group (2000 mg/m^3) exhibited moderately higher total motor activity over the 60-minute time period compared to the females in the control and low exposure groups. For all functional observations, no concentration related effects were reported for the female rats with the exception of fur appearance, where fur condition varied between exposure groups and the high concentration group in which female rats exhibited more occurrences of urine stains (Sterner *et al.*, 2015a). These effects are found in table form in Appendix F.

3.5 Genotoxic Potential

In conjunction with the 90-day study, additional rats (five rats per sex per concentration) were exposed to 0, 200, 700, or 2000 mg/m^3 Gevo ATJ SPK (bio) fuel in an aerosol + vapor mixture for 6 hours per day, 5 days per week for 2 weeks to test for genotoxicity by micronucleus formation (OECD, 1997; U.S. EPA, 1996). Following euthanasia in accordance with current AVMA (2013) guidelines, bone marrow samples collected from femurs were stained and examined for polychromatic erythrocytes, normochromatic erythrocytes, and micronuclei. Exposure-related trends in bone marrow cell toxicity were not observed. The lack of increase in micronuclei across concentrations indicates that Gevo (bio) is not clastogenic (Sterner *et al.*, 2015a).

Two Gevo fuels with additives, Gevo ATJ SPK (non-bio) (POSF 7699) and Gevo ATJ SPK (bio) (POSF 10263) were tested for mutagenic potential using the reverse mutation assay (Ames test), with and without S9 liver microsome metabolic activation (U.S. EPA, 1998d). The assay utilized four strains of *Salmonella typhimurium* (TA-98, TA-100, TA-1535, and TA-1537) and one strain of *Escherichia coli* (WP2) to detect various point mutations induced on the DNA level. Neither fuel was found to be mutagenic (Mumy *et al.*, 2016). Based on this assay and the micronucleus assay, the tested Gevo ATJ SPK fuels are not expected to have mutagenic/genotoxic potential or pose an excess cancer risk when used in an occupational setting. These results are tabulated in Appendix G.

4.0 TOXICITY TESTING OF SWEDISH BIOFUEL ATJ FUELS

Toxicity testing comparison tables for Swedish Biofuel ATJ fuels also are found in Appendices C through G of this document.

4.1 Dermal Irritation

In the same study as discussed in Section 3.1, the two SB ATJ SKA fuels with additives, SB ATJ SKA (old) (POSF 10234) and SB-8 (POSF 8452), were evaluated for dermal irritation potential and compared with results from a petroleum derived JP-8 with additives (POSF 4658+) (Sterner *et al.*, 2014a). Following standardized test guidelines (Draize, 1965; OECD, 2002; U.S. EPA, 1998a), skin areas on New Zealand white rabbits were exposed to each fuel for four hours, with exposure sites either semi-occluded or occluded. Dermal irritation was evaluated at approximately 30 to 60 minutes, and at 24, 48, and 72 hours following exposure, as well as on study days 4, 7, and 14. Semi-occluded exposure to all three jet fuels resulted in scores of "slightly irritating" and occlusion of the test sites raised the dermal irritation index to "moderately irritating" for these three fuels. SB ATJ SKA (old) had been tested for dermal irritation in a prior study by Sterner *et al.* (2014b); both SB ATJ SKA (old) (POSF 10234) and the petroleum-derived JP-8 (POSF 4658+) resulted in "slightly irritating" ratings for both semi-occluded and occluded exposures in the earlier study. These results are tabulated in Appendix C for better comparison. Neither SB ATJ SKA fuel is anticipated to lead to an increase in dermal irritation when handling the fuel in an occupational setting, either alone or in a 50:50 blend with petroleum-derived JP-8.

4.2 Inhalation Toxicity

SB ATJ SKA (new) "SB-8" with additives (POSF 8452) was used in a 90-day inhalation study in conditions very similar to the study with Gevo (bio) (see Section 3.2). Male and female Fischer 344 rats (ten rats per sex per concentration) were exposed to 0, 200, 700 or 2000 mg/m^3 fuel in an aerosol/vapor mixture for 6 hours per day, 5 days per week for 69 exposure days, per standard test guidelines (OECD, 2009; U.S. EPA, 1998b). Average exposure concentrations (aerosol + vapor), measured by FTIR spectrophotometry, were found to be -0.13 ± 0.15, 200.6 ± 2.3, 705.6 ± 13.7, and 2001.8 ± 29.5 mg/m^3 (± SD) in the control, low, medium, and high concentration groups, respectively. The negative concentration for the control chamber was due to instrument drift around zero. Average aerosol concentrations were measured using gravimetric filters and were 1.22 ± 1.36, 1.74 ± 1.4, 3.54 ± 0.76, and 46.6 ± 5.5 mg/m^3 (± SD), for the control, low, medium, and high concentration exposure chambers, respectively. Aerosols represented 0, 0.9, 0.5, and 2.3 percent of the total average jet fuel concentration, respectively; the "aerosol" concentration measured in the control chamber is attributed to background particulates associated with the presence of animals, such as dander, that collected on the filter. Percent body weight gain, measured before and daily during the study, was decreased in the 700 mg/m^3 exposure group males and the 200 mg/m^3 exposure group females at certain intermediate time points during the study; there were no statistical differences for body weight and percent change in body weight for either sex at the end of the study. As there was also a lack of dose-response trend for body weight and body weight gain in both sexes, these statistical differences were not considered biologically significant. Food consumption did not differ between exposure groups throughout the study (Sterner *et al.*, 2015b).

Rats were euthanized in accordance with current (2013) AVMA guidelines on the day following the final exposure. No exposure-related differences were observed in clinical chemistry

endpoints. Hematological changes in exposed male rats were limited to increased platelet counts; counts increased with exposure concentration and were significantly different from the control counts in the 2000 mg/m^3 exposure group. However, in further comparison of platelet counts to historical values provided by the animal supplier (Charles River Laboratories International, Inc., 2009), platelet counts for all male rats in this study (100 percent, control included) were actually lower than the lowest value in the historical count range. The biological significance of the platelet count change is questionable as values for all groups, including the control rats, fell so far outside the range considered to be the biological normal condition. None of the standard hematological parameters were significantly different from the control values in female rats; some female rats in each exposure group had lower platelet counts than the lowest value in the reference range (Charles River Laboratories International, Inc., 2009). Kidney levels of α_{2u}-globulin increased significantly in male rats with increased fuel exposure; all exposed male rats had renal protein levels significantly higher than the control group. Organ weights in exposed animals at necropsy were not different from controls.

Histopathological changes were limited to kidneys and lungs. Minimal focal alveolar epithelial hyperplasia with increased alveolar macrophages was noted in some 2000 mg/m^3 male rats and one female rat of the same exposure group. Early stage CPG was observed in nearly all rats, which is common with increasing age in F344 rats. The severity of findings increased with the higher fuel concentrations only in male rats likely due to species specific male hydrocarbon nephropathy. Overall, the 90-day study with SB-8 with additives resulted in a minimal hyperplasic response in the alveoli for rats in the 2000 mg/m^3 exposure group. Inhalation toxicity results are tabulated in Appendix D.

4.3 Reproductive Health

As an indicator of reproductive health, vaginal cytology was assessed during week 9 of exposure. Sperm count, motility and morphology were evaluated after the full 69 days of exposure. No abnormalities were found for males or females across the exposure groups (see Appendix E).

4.4 Neurobehavioral Effects

Standardized motor activity assays and the functional observational battery were performed during weeks 10 and 11 of exposure, respectively, to assess potential neurobehavioral effects, per U.S. EPA guidelines (1998c). No significant differences were found between exposure groups among the male rats for motor activity or FOB. There were no significant differences in motor activity for female rats, but an increasing trend was noted for total activity time over the 60-minute time period and the corresponding decreasing trend for total resting time. For all functional observations, no concentration related effects were reported for the female rats with the exception of fur appearance. Fur condition varied between exposure groups and the female rats exposed to 700 and 2000 mg/m^3 exhibited more incidences of urine stains in a dose dependent manner (see Appendix F).

4.5 Genotoxic Potential

Additional rats (five rats per sex per concentration) were included at each concentration of SB-8 90-day inhalation toxicity study to assess genotoxicity endpoints (OECD, 1997; U.S. EPA, 1996). These rats received the same aerosol/vapor mixture for 6 hours per day, 5 days per week for 2 weeks. Following euthanasia in accordance with current (2013) AVMA guidelines, bone marrow samples were collected from femurs, stained, and examined for polychromatic erythrocytes, normochromatic erythrocytes and micronuclei per flow cytometry. Neither bone marrow cell toxicity nor an increase in micronuclei were observed; SB-8 is not clastogenic.

Both SB ATJ fuels were tested for mutagenic potential using the reverse mutation assay (Ames test), with and without metabolic activation per guidelines (U.S. EPA, 1998d). The assay utilized four strains of *Salmonella typhimurium* (TA-98, TA-100, TA-1535 and TA-1537) and one strain of *Escherichia coli* (WP2) to detect various point mutations induced on the DNA level. Riccio *et al.* (2010) found no evidence of mutagenicity when testing SB ATJ SKA (old) with additives (POSF 10234). In addition, there was no evidence of mutagenicity with SB-8 with additives (POSF 8452) (Mumy *et al.*, 2016). Based on this assay and the micronucleus assay, the Swedish Biofuel ATJ SKA fuels are not expected to have mutagenic potential or pose an additional cancer risk when used in an occupational setting. All genotoxic assay results are listed in Appendix G.

5.0 DISCUSSION: COMPARISON BETWEEN FUELS

The ATJ fuels manufactured by Gevo and Swedish Biofuels AB share the general chemical processing method which allows these fuels to be built from isobutanol. Due to the ATJ process and uniform starting compound, the composition of each fuel can be tightly controlled per the manufacturers' specifications. However, the resulting fuels are very different from each other. The Gevo fuels, designed to be added to petroleum-derived fuels in mixtures up to 50 percent, contain more than 99 percent aliphatics (Appendix B), whereas the Swedish Biofuels AB fuels were designed to be a drop in replacement for petroleum-derived fuels (SB, 2015) and therefore contain aromatic constituents. This composition difference allows discussion of the contribution of aromatic constituents to the toxicity observed in the studies covered in this report, as well as comparison with other alternative and petroleum-derived jet fuels tested previously.

5.1 Dermal Irritation Comparison

The four ATJ fuels tested for dermal irritation potential resulted in very comparable primary dermal irritation indices (Appendix C). Only the Gevo (non-bio) fuel was considered slightly irritating for both occluded and semi-occluded exposures, making it different from all the other ATJ formulations tested in that occlusion of the exposure site raised the irritation level to moderate for the others. The chemical composition differences between Gevo (non-bio) and the Gevo (bio) ATJ SPK fuels (Appendix B) are slight and do not begin to explain the observed difference in dermal irritation potential. All of the ATJ fuels were tested in the same study; the study design allowed each rabbit to provide multiple randomized test sites and its own control,

minimizing inter-individual differences (Sterner *et al.*, 2014a). However, variation between rabbits may still exist which could lead to a difference in the primary dermal irritation index (PDII) value (Weil and Scala, 1971).

Variation can be seen in the dermal irritation responses for JP-8 (POSF 4658+) as well. The dermal response to JP-8 is typically moderately irritating if occluded and slightly irritating if only semi-occluded (Hurley *et al.*, 2011; Sterner *et al.*, 2014a), but has been judged only slightly irritating in other iterations (Mattie *et al.*, 2013). Although reported by different authors, these studies were conducted in the same laboratory utilizing consistent oversight and training. The variation is therefore likely to occur in the dermal reaction, not in the laboratory methods. Weil and Scala (1971) found that, in general, individual laboratories were able to attain a degree of consistency due to consistent practices; however, the greatest variability existed between the scores for chemicals that are only slightly to moderately irritating. Therefore, although there appear to be some differences in scores between the petroleum-derived JP-8 and all of the alternative fuels listed in Appendix C, the most that can be concluded is that the alternative fuels should not produce increased dermal irritation in DoD personnel handling either the neat alternative fuels or the alternative fuels mixed with petroleum-derived JP-8.

5.2 90-Day Inhalation Toxicity Comparison

Two ATJ fuels, Gevo (bio) ATJ SPK and SB ATJ SKA (new) "SB-8", were tested in 90-day inhalation exposures in rats. In general, few changes were seen in the exposed rats of either group (Appendix D). No biologically significant effect was seen on bodyweight and food consumption (Sterner *et al.*, 2015a and 2015b).

Historically, a dose-dependent decrease in bodyweight is common in animals exposed to high fuel concentrations (Appendix D). Male rats exposed to 500 or 1000 mg/m^3 JP-8 vapor (23 hours/day, continuous exposure, 90 days) weighed 5 or 8 percent less than the control male rats, respectively (Mattie *et al.*, 1991); body weights of female rats in this study were not affected. In a 90-day study with a fuel processed through a hydroprocessed esters and fatty acids (HEFA) method from the Camelina seed (HEFA-C), the 2000 mg/m^3 exposure group average male and female body weights decreased by approximately 5 and 3 percent, respectively (Wong *et al.*, 2013). Rats exposed to 2000 mg/m^3 Fischer-Tropsch (FT) SPK jet fuel were lighter than controls by 12 percent in the males and 5 percent in females; FT exposure also resulted in decreased food consumption among the highest exposure group male and female rats (Mattie *et al.*, 2011a). In the JP-8 study, which was vapor only, the male rat bodyweight effects directly correlated with kidney weight effects likely due to hydrocarbon nephropathy (discussed further below). For the alternative fuels, it is notable that the aerosol exposures were much higher in the HEFA-C and FT fuel exposures than in the ATJ fuel exposures (12 and 33 percent, respectively, versus 2.0 percent for Gevo (bio) and 0.84 percent SB-8, in the highest exposure group for each fuel). The very high aerosol content seen in the FT fuel study may have been a contributor for decreased well-being in this highest exposure group.

Following euthanasia in accordance with current AVMA guidelines (AVMA, 2013), gross pathology indicated no fuel-related differences between exposed and control rats in either ATJ

study; this is consistent with the JP-8 and other alternative fuel studies listed in Appendix D. Organ weights and organ to bodyweight ratios were also evaluated during necropsy. No significant differences were seen in either parameter between exposed and control rats in the SB-8 exposure study (Sterner *et al.*, 2015b). In comparison, some significant changes were seen among rats exposed to the Gevo (bio) fuel, including changes in male spleen and liver weights. As these changes were not dose-related, they were assumed to be due to random biological variability (Sterner *et al.*, 2015a). Similarly, no biologically relevant changes in organ weight were seen in the HEFA-C fuel 90-day study (Wong *et al.*, 2013). Organ weight differences seen at the high dose in the FT study were correlated with body weight decreases and not considered to be a direct effect from the fuel (Mattie *et al.*, 2011a). JP-8 also did not produce changes in organ weights after exposure continuously for 90-days except in the male rat kidneys, where there was a significant increase in hyaline droplet formation (Mattie *et al.*, 1991).

Upon histopathological examination, both fuels were found to have effects in the lungs. SB-8 exposure in the 2000 mg/m^3 group male rats resulted in minimal focal alveolar epithelial hyperplasia with increased alveolar macrophages. Only lung inflammatory infiltrates were recorded among both males and females the 2000 mg/m^3 exposure group in the Gevo (bio) study; this effect appears to be related to decreased clearance of test material droplets in the lungs (Sterner *et al.*, 2015b). The effects seen in the SB-8 study are most similar to the epithelial hyperplasia and lung inflammatory cell infiltration observed in the FT study 2000 mg/m^3 exposure group (Mattie *et al.*, 2011a).

Conversely, however, the SB-8 exposure did not result in any nasal region changes (Sterner *et al.*, 2015b), while in the Gevo (bio) study, mild to moderate irritation-related lesions in the nasal respiratory epithelium occurred in both males and females among the 2000 mg/m^3 exposure group. Although minimal severity lesions were observed in the olfactory epithelium of the nasal cavity in some 700 mg/m^3 exposed rats, these lesions did not appear to exceed background (control) incidence. Mild to moderate intensity lesions were found with frequency only among the 2000 mg/m^3 exposure group. Similarly, minimal to mild goblet cell hyperplasia also occurred only in the 2000 mg/m^3 exposure group. Female rats exhibited more nasal lesions than male rats across both nasal and olfactory epithelium (Sterner *et al.*, 2015a).

Nasal region alterations are a common finding in jet fuel exposures (Appendix D). Similar to the Gevo (bio) study results, olfactory tissue effects (olfactory epithelial degeneration, goblet hyperplasia) were found among rats exposed for 90 days to 2000 mg/m^3 HEFA-C (Wong *et al.*, 2013) and FT SPK (Mattie *et al.*, 2011a) fuels. Increased nasal irritation may be due to the higher aerosol content in the Gevo (bio) study versus the SB-8 study (2.0 versus 0.84 percent aerosol, respectively, in the 2000 mg/m^3 exposure group). In the HEFA-C and FT studies, aerosol exposures are much higher in the 2000 mg/m^3 exposure groups (12 and 33 percent, respectively). Vapor exposures alone do not appear to result in nasal irritation; no histological effects were found in the nasal cavities or lungs of rats exposed to petroleum-derived JP-8 vapor for 90-days continuously (Mattie *et al.*, 1991). Further, an extended time period (90-days) may be required to alter airway histology, as no histological changes were reported in the nasal cavities or lungs after exposure to petroleum-derived Jet A (vapor and aerosol) for only two weeks (Sweeney *et al.*, 2013).

Kidney changes were also noted in the histopathological reports in both studies. Increased age-related chronic progressive glomerulonephropathy was noted for SB-8 and Gevo (bio) exposed male rats; the severity of findings increased with increased fuel exposure in male rats due to hydrocarbon nephropathy. These histopathological changes correlate with a dose-related increase in kidney α_{2u}-globin in these male rats (Sterner *et al.*, 2015a and 2015b). Exposure to the other alternative fuels (FT and HEFA-C fuels) also resulted in dose-dependent increases of α_{2u}-globin measured in the kidney (Mattie *et al.*, 2011a; Wong *et al.*, 2013). Petroleum-derived JP-8 strongly induces the production of this protein (Mattie *et al.*, 1991). Chronic hydrocarbon exposure stimulates α_{2u}-globin production, leading to increased hyaline droplet formation, ultimately resulting in renal tubule tumors following long-term exposure (Bruner et al., 1993). However, this process is observed only in the male rat, and is considered not applicable to human toxicology. The degree of formation appears to be dependent on the hydrocarbons present in the fuel, although a simple fuel comparison (Appendix B) does not provide a conclusive answer as to which hydrocarbons result in a higher formation of α_{2u}-globin.

Male rats exposed to 2000 mg/m^3 SB-8 did demonstrate a significant increase in platelet counts (Sterner *et al.*, 2015b). Although this increase was clearly dose-dependent, its significance is unknown as all platelet accounts in the male rat groups (control included) were less than the lowest value in the reference range provided by the animal supplier (Charles River Laboratories International, Inc., 2009). No standard hematologic parameters were altered significantly among the SB-8 or the Gevo (bio) exposed female rats (Sterner *et al.*, 2015a). A dose-dependent increase in WBC counts was found among male rats exposed to Gevo (bio); although the WBC counts were significantly higher than controls among the 2000 mg/m^3 Gevo (bio) exposed males, the values fell within normal ranges (Charles River Laboratories International, Inc., 2009) and were not considered biologically significant. No biologically significant changes attributable to exposure have been identified in the clinical chemistry and hematologic analyses for male or female rats exposed to JP-8 (Mattie *et al.*, 1991), FT (Mattie *et al.*, 2011a), or HEFA-C (Wong *et al.*, 2013) during their respective 90-day inhalation studies.

Reproductive health, screened through vaginal cytology and sperm motility, mobility, morphology, was not impacted by either fuel (Appendix E; Sterner *et al.*, 2015a and 2015b). Similarly, neither HEFA-C nor FT fuels altered reproductive health as measured by these same parameters. Mattie *et al.* (2000) reported the results of reproductive toxicity studies in which rats were exposed via oral administration of JP-8. In the first study, male rats were given 0, 750, 1500, or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naïve females to assess fertility and sperm parameters. After 70 days of dosing, body weights in the 3000 mg/kg group were over 30 percent lower than control weights. In the second reproductive study, general toxicity, fertility and reproductive endpoints were assessed in female rats dosed with neat JP-8 (0, 325, 750, or 1500 mg/kg) daily by gavage for a total of 21 weeks (90-days plus mating with naïve males, gestation, and lactation); the female rats also displayed significant dose-dependent decreases in body weights. Notably, neither the male-only nor the female-only 90-day oral exposures to JP-8 altered pregnancy rate, gestation length, or number of pups per litter. The female-only exposure did result in decreased pup weights in the highest dose group, which were determined to be directly related to decreased maternal weight and therefore not considered a reproductive-specific effect. Although complete multi-generation reproductive and

developmental studies have not been performed with JP-8 or its alternative fuels, there is no indication that reproductive specific effects would be anticipated.

Neurobehavioral function was assessed in both ATJ studies (Appendix F). No changes were noted in the male rats in either study. For female rats, motor activity measurements increased among the 2000 mg/m^3 exposed groups; the high exposure group females exposed to Gevo (bio) displayed greater total activity during the 60 minute assessment, and those exposed to SB-8 exhibited more rearing behaviors over the 60 minutes. Both female high exposure groups were noted to have more urine stains on their fur, reflecting a failure to groom normally, during the course of the functional observational battery (Sterner *et al.*, 2015a and 2015b). Overall, the female rats displayed limited behavioral changes when exposed to 2000 mg/m^3 of either fuel.

In contrast to the ATJ findings, minor but opposite neurobehavioral findings were reported during the FT 90-day inhalation study. Male rats exposed to 2000 mg/m^3 FT fuel showed a reduction in total activity. Female rats of the same group displayed reduced initial exploratory activity. FOB findings were limited to reduced rearing behavior in females exposed to 2000 mg/m^3 FT fuel (Mattie *et al.*, 2011a). During the HEFA-C 90-day study, there were no significant observations relatable to exposure to HEFA-C fuel in either motor activity measurements or the FOB (Wong *et al.*, 2013). Alternative fuels can produce neurobehavioral effects, but of a limited scope at the highest tested concentrations.

Although multiple neurobehavioral studies have been performed to determine JP-8 effects, none were identical to the motor activity and FOB assessments performed in this study. Changes in behavioral response were observed in two studies where rats were exposed to 0, 500, or 1000 mg/m^3 JP-8 vapor only for 6 hours/day 5 days a week for 6 weeks. The rats scored the same as control animals on all simple learning tasks including lever acquisition and lever spatial reversal tasks. On moderate to difficult tasks such as incremental repeated acquisition, the lower exposure group animals demonstrated better performance than high dose animals; neither group performed differently from controls using statistical analysis (Ritchie *et al.*, 2001). In a second study using the same exposure methods, animals were tested in a large battery of neurobehavioral tasks. No exposure group differences were found in acoustic startle responses, forelimb grip strength, nociception, social interaction, the forced swim test, spontaneous locomotor activity, passive avoidance or Morris water maze performance. However, animals exposed to JP-8 spent more time than control animals investigating the appetitive stimulus, suggesting behavioral sensitization and altered neural pathways related to the dopaminergic system (Rossi *et al.*, 2001). Overall, only two specific neurobehavioral effects of JP-8 vapor were seen after exposure in adult rats.

5.3 Mutagenicity Potential Comparison

ATJ fuels were tested for mutagenicity and genotoxicity using two different assays (Appendix G). The reverse mutation assay or Ames test was used to screen four ATJ fuels for mutagenic potential. Each fuel was screened using a standard procedure against four *Salmonella typhimurium* strains and one strain of *Escherichia coli*; assays were performed with and without

metabolic activation. All four ATJ fuels were found to be negative for mutagenicity using this assay (Mumy *et al.*, 2016; Riccio *et al.*, 2010).

Similarly, HEFA-C and HEFA from a tallow feedstock (rendered beef fat, HEFA-T) were also established as non-mutagenic using this assay by Mattie *et al.* (2013). Riccio *et al.* (2010) found HEFA from mixed fats and oils (HEFA-F) to be negative for mutagenicity in the Ames test; HEFA-F was referred to in this study as R-8 or renewable JP-8 (POSF 5469). FT was also shown to be non-mutagenic by the Ames assay in two studies (Mattie *et al.*, 2011b; Riccio *et al.*, 2010). The Ames assay was a relatively new procedure in the 1970s when Brusick and Matheson (1978) demonstrated that JP-8 was not mutagenic.

The mammalian micronucleus assay was performed in conjunction with each 90-day study. Groups of five rats were exposed for two weeks alongside the 90-day study rats. Femoral bone marrow cells were examined for micronucleated reticulocytes, a marker of genotoxicity, and reticulocytes were quantified as indicators of bone marrow toxicity (decreased reticulocytes) or immune response (increased reticulocytes). Neither SB-8 nor Gevo (bio) fuel exposure resulted in significant changes in this assay (Sterner *et al.*, 2015a and 2015b). The alternative fuels FT and HEFA-C were also found to be non-genotoxic using the micronucleus assay conducted with identical methodology as the ATJ fuels (Mattie *et al.*, 2011a; Wong *et al.*, 2013). FT was also negative in a chromosomal aberration assay in which human lymphocytes were exposed to the fuel *in vitro* (Mattie *et al.*, 2011b).

In a dermal variation of the mammalian micronucleus test, mice were treated with either a single or multiple applications of JP-8 and Jet A fuels. Using several different dermal exposure regimens, no statistically significant differences in the incidence of reticulocytes or micronucleated reticulocytes were observed in the bone marrow and/or peripheral blood of mice treated with JP-8 or Jet-A when compared with those of untreated control animals (Vijayalaxmi *et al.*, 2006; Vijayalaxmi, 2011). Based on these results, ATJ alternative fuels, other tested alternative fuels and petroleum-derived JP-8 are not expected to have mutagenic potential or pose an additional cancer risk when used in an occupational setting.

6.0 CONCLUSIONS AND OEL RECOMMENDATION

The Gevo ATJ fuels have been approved by ASTM in a mixture with petroleum-derived fuels up to 30 percent at present (ASTM, 2016). Future use of the alternative fuels in DoD is uncertain due to comparative pricing with oil. However, ATJ SPK and SKA fuels such as Gevo (bio) and SB-8 were found to have similar toxicity or somewhat lower toxicity than petroleum-derived JP-8 in use by the DoD today. Handling of ATJ fuels alone or in a blend with petroleum-derived JP-8 is unlikely to increase human health risks in the military workplace. Therefore, the occupational exposure level for both the Gevo (bio) ATJ SPK and the SB ATJ SKA (new) "SB-8" fuel is recommended to remain the same as for JP-8: 200 mg/m^3 (vapor) and 5 mg/m^3 (aerosol) (NRC, 2003).

7.0 REFERENCES

ASTM. 2016. Standard Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbons. West Conshohocken PA: ASTM International. Designation: D7566-16.

AVMA. 2013. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. Schaumburg IL: American Veterinary Medical Association. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf.

Borghoff, S.J., Short, B.G. and Swenberg, J.A. 1990. Biochemical mechanisms and pathobiology of alpha $_{2u}$-globulin nephropathy. Annu. Rev. Pharmacol. Toxicol. 30: 349-367.

Bruner, R.H., Kinkead, E.R., O'Neill, T.P., Flemming, C.D. Mattie, D.R., Russell, C.A., and Wall, H.G. 1993. The Toxicologic and Oncogenic Potential of JP 4 Jet Fuel Vapors in Rats and Mice: 12 Month Intermittent Inhalation Exposure. Fundam Appl Toxicol. 20:97-110.

Brusick, D.J. and Matheson, D.W. 1978. Mutagen and oncogen study on JP-8. Wright-Patterson AFB, OH: Aerospace Medical Research Laboratory. AMRL-TR-78-20.

Charles River Laboratories International, Inc. 2012. Technical Sheet: CDF (Fischer) Rat Hematology, North American Colonies, January 2009 – December 2009. Wilmington MA. <http://www.criver.com/files/pdfs/rms/fischer-cdf/rm_rm_r_cdf_fisher_rat_clinical_pathology_data.aspx>

Draize, J.H. 1965. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity. Topeka, KS: Association of Food and Drug Officials of the United States. pp. 49-52.

Gevo. 2011. Transportation Fuels. Gevo White Paper. http://www.gevo.com/assets/pdfs/GEVO-wp-iso-ftf.pdf. Accessed 11 May 2015.

Hard, G.C., Rodgers, I.S., Baetcke, K.P., Richards, W.L., McGaughy, R.E. and Valcovic, L.R. 1993. Hazard evaluation of chemicals that cause accumulation of alpha $_{2u}$-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ. Health Perspect. 99: 313-349.

Hurley, J.M., Wagner, D., Sterner, T.R. and Mattie, D.R. 2011. Acute dermal irritation study of JP-8 and S-8 in New Zealand white rabbits. Wright-Patterson AFB, OH: Air Force Research Laboratory, Applied Biotechnology Branch. AFRL-RH-WP-TR-2011-0054, ADA546698.

Mattie, D.R., Alden, C.L., Newell, T.K., Gaworski, C.L., Flemming, C.D.1991. A 90-day continuous vapor inhalation toxicity study of JP-8 jet fuel followed by 20 or 21 months of recovery in Fischer 344 rats and C57BL/6 mice. Toxicol Pathol. 19(2):77-87.

Mattie, D.R., Hurley, J.M., Riccio, E.S. and Sterner, T.R. 2013. Acute Dermal Irritation Study and *Salmonella-Escherichia coli*/Microsome Plate Incorporation Assay of Hydroprocessed Esters and Fatty Acids (HEFA) Bio-Based Jet Fuels. Wright-Patterson AFB OH: Air Force Research Laboratory, 711th Human Performance Wing, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffects Branch. AFRL-RH-WP-TR-2012-0011, ADA584649.

Mattie, D.R., Marit, G.B., Cooper, J.R., Sterner, T.R., Flemming, C.D. 2000. Reproductive effects of JP-8 jet fuel on male and female Sprague-Dawley rats after exposure by oral gavage. Air Force Research Laboratory, Human Effectiveness Directorate, Wright-Patterson AFB, OH. AFRL-HE-WP-TR-2000-0067, ADA453146.

Mattie, D.R., Sterner, T.R., Oppong-Nketiah, M., Becker, T., Wallner, B., Riedel, W., Lutterbach, A. and Wagner, D.J. 2011b. F-T jet fuel reverse mutation assay and chromosome aberration test. Wright-Patterson AFB, OH: Air Force Research Laboratory, 711 Human

Performance Wing, Applied Biotechnology Branch. AFRL-RH-WP-TR-2011-0010, ADA536685.
Mattie, D.R., Sterner, T.R., Wong, B.A., Dodd, D.E., Layko, D.K., Ross, P.W., Gross, E.A., Willson, G.A., Hinz, J.P. and Wagner, D.J. 2011a. 90-Day inhalation toxicity study of FT fuel. Wright-Patterson AFB, OH: Air Force Research Laboratory, 711 Human Performance Wing, Human Effectiveness Directorate, Molecular Bioeffects Branch. AFRL-RH-FS-2011-0014, ADA557260.
Milbrandt, C., Kinchin, C. and McCormick, R. 2013. The Feasibility of Producing and Using Biomass-Based Diesel and Jet Fuel in the United States. Golden CO: National Renewable Energy Laboratory. NREL/TP-6A20-58015.
Mumy, K.L., Gargas, N.M., Lemmer, G.P., McInturf, A.M., Hulgan, A.D. and Mattie, D.R. 2016. Evaluation of 10 Jet Fuels in the *Salmonella-Escherichia coli* Mutagenicity Assay. Wright-Patterson AFB, OH: Naval Medical Research Unit Dayton (NAMRU-D). NAMRU-D-16-101.
NRC. 2003. Toxicologic Assessment of Jet-Propulsion Fuel 8. National Research Council of the National Academies of Science, Committee on Toxicology. National Academy Press, Washington, D.C.
OECD. 1997. OECD guideline for testing of chemicals: *In vitro* mammalian chromosome aberration test. OECD Guideline 473. Organisation for Economic Co-operation and Development, Paris.
OECD. 2002. OECD guideline for the testing of chemicals. Guideline 404: Acute Dermal Irritation/Corrosion. Organisation for Economic Co-operation and Development, Paris.
OECD. 2009. OECD guideline for testing of chemicals. Guideline 413: Subchronic inhalation toxicity: 90-day study. Organisation for Economic Co-operation and Development, Paris.
Riccio, E.S., Green, C.E. and Mattie, D.R. 2010. Evaluation of five jet fuels in the *Salmonella-Escherichia coli* / microsome plate incorporation assay. Wright-Patterson AFB, OH: Air Force Research Laboratory, 711 Human Performance Wing, Applied Biotechnology Branch. AFRL-RH-WP-TR-2010-0138, ADA536581.
Ritchie, G.D., Rossi, J. III, Nordholm, A.F., Still, K.R., Carpenter, R.L., Wenger, G.R., Wright, D.W. 2001. Effects of repeated exposure to JP-8 jet fuel vapor on learning of simple and difficult operant tasks by rats. J Toxicol Environ Health A. 64(5):385-415.
Rossi, J. III, Nordholm, A.F., Carpenter, R.L., Ritchie, G.D., Malcomb, W. 2001. Effects of repeated exposure of rats to JP-5 or JP-8 jet fuel vapor on neurobehavioral capacity and neurotransmitter levels. J Toxicol Environ Health A. 63(6):397-428.
Satyarthi, J.K., Thota, C., Shastry, G.D.T. and Viswanathan, P.S. 2013. Options for Biojet Fuel. Petroleum Technology Quarterly 18. http://www.digitalrefining.com/article_1000793.pdf.
SB. 2015. Swedish BioFuels. http://www.swedishbiofuels.se/ Accessed 12 March 2015.
Sterner, T.R., Hurley, J.M. and Mattie, D.R. 2014b. Acute Dermal Irritation Study of Ten Jet Fuels in New Zealand White Rabbits: Comparison of Synthetic and Bio-Based Jet Fuels with Petroleum JP-8 Wright-Patterson AFB OH: Air Force Research Laboratory, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffects Branch. AFRL-RH-WP-TR-2014-0045, ADA606124.
Sterner, T.R., Hurley, J.M., Edwards, J.T., Shafer, L.M. and Mattie, D.R. 2014c. Acute Dermal Irritation Study of Six Jet Fuels in New Zealand White Rabbits: Comparison of Four Bio-Based Jet Fuels with Two Petroleum JP-8 Fuels. Wright-Patterson AFB OH: Air Force

Research Laboratory, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffects Branch. AFRL-RH-WP-TR-2014-0046, ADA606443.
Sterner, T.R., Hurley, J.M., Shafer, L.M., Striebich, R.C., and Mattie, D.R. 2014a. Acute Dermal Irritation Study in New Zealand White Rabbits: Four Alcohol-to-Jet (ATJ) Synthetic Paraffinic Kerosene (SPK) Alternative Jet Fuels Compared with Petroleum JP-8. Wright-Patterson AFB OH: Air Force Research Laboratory, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffects Branch. AFRL-RH-WP-TR-2014-0133.
Sterner, T.R., Wong, B.A., Mumy, K.L., McInturf, S.M., Gargas, N.M., Reboulet, J.E., James, R.A., Stoffregen, D.E. and Mattie, D.R. 2015b. 90-Day Inhalation Toxicity Study of Swedish Biofuel Alcohol-to-Jet (ATJ) Synthetic Kerosene with Aromatics (SPA) in Rats with Neurotoxicity Testing and Genotoxicity Assay. Wright-Patterson AFB OH: Air Force Research Laboratory, 711th Human Performance Wing, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffects Branch. AFRL-RH-FS-TR-2015-, ADA627833.
Sterner, T.R., Wong, B.A., Mumy, K.L., McInturf, S.M., Grimm, M.D., Gargas, N.M., Stoffregen, D.E., Mattie, D.R. 2015a. 90-Day Inhalation Toxicity Study of Bio-Derived Gevo Alcohol-To-Jet (ATJ) Synthetic Paraffinic Kerosene (SPK) in Rats with Neurotoxicity Testing and Genotoxicity Assay. Wright-Patterson AFB OH: Air Force Research Laboratory, 711th Human Performance Wing, Human Effectiveness Directorate, Bioeffects Division, Molecular Bioeffects Branch. AFRL-RH-FS-TR-2015-0031, ADA623546.
Sterner, T.R., Sweeney, L.M., Mumy, K.L., Wong, B.A., James, R.A., Reboulet, J., Sharits, B., Grimm, M., Gargas, N., Striebich, R.C. and Mattie, D.R. 2013. Hydroprocessed Esters and Fatty Acids (HEFA) Bio-Based Jet Fuels: Sensory Irritation Study and Human Health Hazard Assessment. Wright-Patterson AFB OH: Air Force Research Laboratory, 711th Human Performance Wing, Human Effectiveness Directorate, Molecular Bioeffects Branch. AFRL-RH-FS-TR-2014-0001, ADA595854.
Striebich, R.C., Shafer, L.M., Adams, R.K., West, Z.J., DeWitt, M.J., Zabarnick, S. 2014. Hydrocarbon Group-Type Analysis of Petroleum-Derived and Synthetic Fuels Using Two-Dimensional Gas Chromatography. Energy Fuels. 28:5696-5706.
Sweeney, L.M., Phillips, E.A., Gut, C.P., Okolica, M.R., Reboulet, J.E. 2013. Toxicokinetic study for investigation of sex differences in internal dosimetry of jet propulsion fuel 8 (JP-8) in the laboratory rat. Naval Medical Research Unit-Dayton ,Wright-Patterson AFB OH.
U.S. EPA. 1996. Health effects test guidelines: OPPTS 870.5395 *In vivo* mammalian cytogenetics tests: Erythrocyte micronucleus assay. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 712-C-96-226.
U.S. EPA. 1998a. Health effects test guidelines: OPPTS 870.2500 Acute dermal irritation. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 712-C-98-196.
U.S. EPA. 1998b. Health effects test guidelines: OPPTS 870.3465 90-Day inhalation toxicity. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 712-C-98-204.
U.S. EPA. 1998c. Health effects test guidelines. OPPTS 870.6200 Neurotoxicity screening battery. Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C. EPA 712-C-98-238.

U.S. EPA. 1998d. Health effects test guidelines: OPPTS 870.5100 Bacterial reverse mutation test. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 712-C-98-247.

Vijayalaxmi, V., Kligerman, A.D., Prihoda, T.J., Ullrich, S.E. 2006. Micronucleus studies in the peripheral blood and bone marrow of mice treated with jet fuels, JP-8 and Jet-A. Mutat Res. 608(1):82-87.

Vijayalaxmi. 2011. Genetic damage in the blood and bone marrow of mice treated with JP-8 jet fuel. In: Jet Fuel Toxicology. Edited by Witten ML, Zeiger E, Ritchie GD. New York: CRC Press; 2011: 239-245.

Weil, C.S. and Scala, R.A. 1971. Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests. Toxicol Appl Pharmacol. 19:276-360.

Wong, B.A., Howard, W.R., Sterner, T.R., Elliott, M.W. and Mattie, D.R. 2013. 90-Day Inhalation Toxicity Study of Hydroprocessed Esters and Fatty Acids (HEFA) Bio-Based Jet Fuel in Rats with Neurotoxicity Testing and Genotoxicity Assay. Wright-Patterson AFB OH: Air Force Research Laboratory, 711th Human Performance Wing, Molecular Bioeffects Branch. AFRL-RH-WP-TR-2013-0109, ADA596005.

APPENDIX A. FUELS, POSF NUMBERS AND MANUFACTURER INFORMATION

This list is comprised of fuels discussed in the current report. The required additive package is a proprietary mixture which includes an anti-corrosion agent and a fuel system icing inhibitor; it is added at the same percentage by volume across petroleum-derived and alternative fuels.

Use of commercial names and products does not constitute endorsement of these products by the U.S. Air Force, U.S. Navy, or U.S. Army.

FUEL CLASS	**FUEL NAME**	**POSF** (neat)	**POSF** (with additives)	**Manufacturer and Notes**
Alcohol to Jet Synthetic Paraffinic Kerosene	Gevo (non-bio) ATJ SPK	7695	7699	Gevo (Englewood CO) Made from petrochemicals
	Gevo (bio) ATJ SPK	9641	10021	Gevo (Englewood CO) Made from biologically derived feedstocks
		10262	10263	Gevo (Englewood CO) Made from biologically derived feedstocks; Chemically identical to POSF 9641
Alcohol to Jet Synthetic Kerosene with Aromatics	SB ATJ SKA (old)	5668	10234	Swedish Biofuels AB (Stockholm) Contains only trimethylbenzenes in aromatic fraction
	SB ATJ SKA (new) "SB-8"	7633	8452	Swedish Biofuels AB (Stockholm) Aromatic fraction contains several alkylbenzene components
Hydroprocessed Esters and Fatty Acids Synthetic Paraffinic Kerosene	HEFA-C SPK (Camelina)	6152	6183	UOP LLC, a Honeywell Company (Des Plaines IL)
	HEFA-F SPK (mixed fats and oils); R-8	5469	5480	Syntroleum Corporation (Tulsa OK)
	HEFA-T SPK (tallow)	6308	6346	UOP LLC, a Honeywell Company (Des Plaines IL)
Fischer-Tropsch Synthetic Paraffinic Kerosene	FT SPK; S-8	4734	5109	Syntroleum Corporation (Tulsa OK)
Petroleum-derived Jet Fuel	JP-8	4658	4658+	Reference Jet A fuel blended at AFRL/RQTF from equal volumes of 5 Jet A fuels representing sources across the U.S.A.
	JP-8	3404	3509	Jet A fuel blended at AFRL/RQTF from equal volumes of 5 refinery streams (3 suppliers, different petrochemical processes) to form a representative fuel

APPENDIX B. SUMMARY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COMPONENT COMPARISON TABLE

Table values excerpted from the references cited; more detailed analyses of the fuel constituents as determined by comprehensive two-dimensional gas chromatography can be found in those references. Component values are given in mass percent.

FUEL	Gevo ATJ SPK (non-bio)	Gevo ATJ SPK (bio)	SB ATJ SKA (old)	SB ATJ SKA (new) "SB-8"	JP-8
POSF (neat fuel)	7695	9641	5668	7633	4658
AROMATICS					
Total Alkylbenzenes	0.24	<0.01	13.16	10.47	13.69
Total Alkylnaphthalenes	<0.01	<0.01	<0.01	<0.01	1.76
Total Cycloaromatics	0.01	<0.01	0.02	1.71	5.79
Total Aromatics	**0.25**	**0.01**	**13.18**	**12.18**	**21.24**
ALIPHATICS					
Total iso-Paraffins	98.27	99.43	86.30	44.14	31.34
Total n-Paraffins	0.02	0.02	0.05	0.66	19.00
Total Cycloparaffins	1.47	0.54	0.47	43.00	28.42
Total Aliphatics	**99.76**	**99.99**	**86.82**	**87.80**	**78.76**
REFERENCE	Sterner *et al.* (2014a)				

FUEL	FT SPK	HEFA-C SPK	HEFA-T SPK	HEFA-F SPK
POSF (neat fuel)	4734	6152	6308	5469
AROMATICS				
Total Alkylbenzenes	0.09	0.26	0.20	0.46
Total Alkylnaphthalenes	0.03	<0.01	0.02	<0.01
Total Cycloaromatics	0.05	0.06	0.09	0.10
Total Aromatics	**0.16**	**0.33**	**0.31**	**0.56**
ALIPHATICS				
Total iso-Paraffins	79.90	84.18	87.21	81.70
Total n-Paraffins	19.11	11.41	11.61	14.62
Total Cycloparaffins	0.82	4.09	0.86	3.12
Total Aliphatics	**99.84**	**99.67**	**99.69**	**99.44**
REFERENCE	Sterner *et al.* (2014c)	Sterner *et al.* (2013)		

APPENDIX C. DERMAL IRRITATION COMPARISON TABLE

Table values excerpted from the references cited. The FT-SPK/JP-8 mixture was created using equal volumes as noted by the designation vol/vol (volume/volume). PDII refers to primary dermal irritation index.

FUEL POSF	**Exposure**	**PDII**	**Descriptive Rating**	**Reference**
Gevo (non-bio) ATJ SPK 7699	Occluded	1.6	Slightly Irritating	Sterner *et al.* (2014a)
	Semi-Occluded	1.0		
Gevo (bio) ATJ SPK 10021	Occluded	2.4	Moderately Irritating	
	Semi-Occluded	1.0	Slightly Irritating	
SB ATJ SKA (old) 10234	Occluded	2.3	Moderately Irritating	
	Semi-Occluded	1.2	Slightly Irritating	
SB ATJ SKA (new) "SB-8" 8452	Occluded	2.2	Moderately Irritating	
	Semi-Occluded	1.3	Slightly Irritating	
JP-8 4658+	Occluded	2.5	Moderately Irritating	
	Semi-Occluded	1.1	Slightly Irritating	
JP-8 4658+	Occluded	0.8	Slightly Irritating	Mattie *et al.* (2013)
	Semi-Occluded	0.8		
HEFA-C SPK 6152	Occluded	0.9	Slightly Irritating	
	Semi-Occluded	0.6		
HEFA-T SPK 6308	Occluded	0.6	Slightly Irritating	
	Semi-Occluded	0.2		
HEFA-F SPK 5469	Occluded	0.3	Slightly Irritating	
	Semi-Occluded	0	Nonirritating	
JP-8 4658+	Occluded	2.1	Moderately Irritating	Hurley *et al.* (2011)
	Semi-Occluded	1.8	Slightly Irritating	
FT SPK 5109	Occluded	2.3	Moderately Irritating	
	Semi-Occluded	0.8	Slightly Irritating	
JP-8/FT 50/50 mixture (vol/vol)	Occluded	1.9	Slightly Irritating	
	Semi-Occluded	1.5		

APPENDIX D. 90-DAY INHALATION TOXICITY COMPARISON TABLE

Table values excerpted from the references cited. Abbreviations used in the table include: dy = day, F = female, hr = hour, LOAEC = lowest observed adverse effect concentration, M = male, NOAEC = no observed adverse effect concentration, NR = not reported, wk = week

FUEL POSF	**Exposure** mg/m^3 (% aerosol)	**Exposure Duration**	**NOAEC LOAEC** mg/m^3	**Result**	**Reference**
Gross Observations					
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk	2000 no LOAEC	No significant dose-related changes	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)	6 hr/dy, 5 dy/wk	2000 no LOAEC		Sterner *et al.* (2015b)
JP-8 0463	0, 500, 1000 (vapor only)	23 hr/dy continuous	vapor: 500 vapor: 1000	-- Decreased body weight (M only, 8%)	Mattie *et al.* (1991)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk	700 2000	-- Decreased body weight (M 5%, F 3%)	Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)	6 hr/dy, 5 dy/wk	700 2000	-- Decreased body weight (M 12%, F 5%) Decreased food consumption (M & F)	Mattie *et al.* (2011)

FUEL POSF	**Exposure** mg/m^3 (% aerosol)	**Exposure Duration**	**NOAEC LOAEC** mg/m^3	**Result**	**Reference**
Gross Pathology, Histopathology					
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk	700 2000	-- Increased nasal respiratory epithelial lesions (irritation related), olfactory epithelial lesions (minimal severity) and goblet cell hyperplasia (minimal to mild severity); Increased lung inflammatory infiltrates (clearance deficits) (M&F) Increased chronic progressive glomerulo-nephropathy (M)	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)	6 hr/dy, 5 dy/wk	700 2000	-- Minimal Type II pneumocyte hyperplasia (M) Alveolar histiocytosis leading to hyperplasia (M & F) Increased chronic progressive glomerulo-nephropathy (M) Mild Harderian gland inflammation (mild eye irritation) (M & F)	Sterner *et al.* (2015b)
JP-8 0463	0, 500, 1000 (vapor only)	23 hr/dy continuous	NA to humans (male rat specific)	Increased relative kidney weight & kidney lesions due to hydrocarbon nephropathy (M)	Mattie *et al.* (1991)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk	700 2000	-- Olfactory epithelial degeneration & goblet hyperplasia in nasal airways (M & F)	Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)	6 hr/dy, 5 dy/wk	700 2000	-- Olfactory epithelial degeneration & respiratory epithelial hyperplasia in nasal airways; Lung inflammatory cell infiltration (M & F)	Mattie *et al.* (2011b)

FUEL POSF	**Exposure** mg/m^3 (% aerosol)	**Exposure Duration**	**NOAEC LOAEC** mg/m^3	**Result**	**Reference**
Clinical Chemistry, Hematology					
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk	700 2000	-- Increased total white blood cells (M only)	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)		700 2000	-- Increased platelets (M)	Sterner *et al.* (2015b)
JP-8 0463	0, 500, 1000 (vapor only)	23 hr/day continuous	vapor: 1000 no LOAEC	No dose-dependent changes in clinical chemistry or hematology	Mattie *et al.* (1991)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk	2000 no LOAEC	No dose-dependent changes in clinical chemistry or hematology	Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)		2000 no LOAEC		Mattie *et al.* (2011b)
Alpha$_{2u}$-Globulin					
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk	not applicable to humans	Dose-dependent increase in kidney α_{2u}-globulin concentration (M)	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)	6 hr/dy, 5 dy/wk			Sterner *et al.* (2015b)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk			Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)	6 hr/dy, 5 dy/wk			Mattie *et al.* (2011b)

APPENDIX E. REPRODUCTIVE HEALTH COMPARISON TABLE

Table values excerpted from the references cited. *Units are mg/m^3 unless stated differently within the row for oral studies. Abbreviations used in the table include: dy = day, F = female, hr = hour, LOAEC/LOAEL = lowest observed adverse effect concentration/level, M = male, NOAEC/NOAEL = no observed adverse effect concentration/level, NR = not reported, wk = week

FUEL POSF	**Exposure** *mg/m^3 (% aerosol)	**Exposure Duration**	**NOAEC LOAEC** *mg/m^3	**Result**	**Reference**
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk, 90 dy	2000 no LOAEC	No significant differences in male reproductive endpoints	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)		2000 no LOAEC	No alteration of estrus cycle (vaginal cytology endpoints)	Sterner *et al.* (2015b)
JP-8 NR	ORAL (0, 750, 1500, 3000 mg/kg)	M ONLY 1 dose/dy, 90 dy	3000 mg/kg (NOAEL) no LOAEL	No significant changes for pregnancy rate, gestation length, sperm parameters	Mattie *et al.* (2000)
JP-8 NR	ORAL (0, 325, 750, 1500 mg/kg)	F ONLY 1 dose/dy, 146 dy	1500 mg/kg (NOAEL) no LOAEL	No significant changes for gestation length, pregnancy rate, number of pups/litter	Mattie *et al.* (2000)
			750 mg/kg (NOAEL) 1500 mg/kg (LOAEL)	-- Decreased pup weight related to decreased maternal weight	
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk, 90 dy	2000 no LOAEC	No significant differences in male reproductive endpoints	Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)		2000 no LOAEC	No alteration of estrus cycle (vaginal cytology endpoints)	Mattie *et al.* (2011a)

APPENDIX F. NEUROBEHAVIORAL EFFECTS COMPARISON TABLE

Table values excerpted from the references cited. Abbreviations used in the table include: dy = day, F = female, hr = hour, LOAEC = lowest observed adverse effect concentration, M = male, NOAEC = no observed adverse effect concentration, wk = week

FUEL	Exposure *mg/m^3 (% aerosol)	Exposure Duration	NOAEC LOAEC *mg/m^3	Result	Reference
Functional Observation Battery					
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk	700 2000	-- Urine stained fur (F only)	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)	6 hr/dy, 5 dy/wk	700 2000	-- Urine stained fur (F only)	Sterner *et al.* (2015ba)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk, 90 dy	2000 no LOAEC	No significant changes (M & F)	Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)	6 hr/dy, 5 dy/wk, 90 dy	700 2000	-- Reduced rearing behavior (F)	Mattie *et al.* (2011a)
Motor Activity					
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk	700 2000	-- Increased total distance/60 min (F only)	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)	6 hr/dy, 5 dy/wk	700 2000	-- Increased total number of rears/60 min (F only)	Sterner *et al.* (2015b)
JP-8 3509	70, 500, 1000 (vapor only)	6 hr/dy, 5 dy/wk, 90 dy	vapor: 1000 no LOAEC	No significant changes (M & F)	Rossi *et al.* (2001)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)	6 hr/dy, 5 dy/wk, 90 dy	2000 no LOAEC	No significant changes (M & F)	Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)	6 hr/dy, 5 dy/wk	700 2000	-- Reduced total activity (M)	Mattie *et al.* (2011a)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)	6 hr/dy, 5 dy/wk, 90 dy	700 2000	-- Reduced initial exploratory activity (F)	Mattie *et al.* (2011a)

FUEL	Exposure *mg/m^3 (% aerosol)	Exposure Duration	NOAEC LOAEC *mg/m^3	Result	Reference
Other Neurobehavioral Tests					
JP-8 3509	70, 500, 1000 (vapor only)	6 hr/dy, 5 dy/wk, 90 dy	vapor: 500 vapor: 1000	-- Deficits in acquisition, performance of moderate to difficult tasks (stimulus reversal, incremental repeated acquisition)	Ritchie *et al.* (2001)
JP-8 3509	70, 500, 1000 (vapor only)	6 hr/dy, 5 dy/wk, 90 dy	vapor: 1000 no LOAEC	No difficulty with simple learning tasks (lever acquisition, fixed ratio, lever spatial reversal)	Ritchie *et al.* (2001)
JP-8 3509	70, 500, 1000 (vapor only)	6 hr/dy, 5 dy/wk, 90 dy	vapor: 1000 no LOAEC	No difficulty with acoustic startle, forelimb grip strength, nociception, social interaction, forced swim test, passive avoidance, Morris water maze	Rossi *et al.* (2001)
JP-8 3509	70, 500, 1000 (vapor only)	6 hr/dy, 5 dy/wk, 90 dy	vapor: 500 vapor: 1000	-- Longer duration spent on appetitive stimulus approach sensitization assay	Ritchie *et al.* (2001)

APPENDIX G. GENOTOXIC POTENTIAL COMPARISON TABLE

Table values excerpted from the references cited. Abbreviations used in the table include: dy = day, F = female, hr = hour, LOAEC = lowest observed adverse effect concentration, M = male, NOAEC = no observed adverse effect concentration, wk = week

Rat *In Vivo* Erythrocyte Micronucleus Test

FUEL POSF	**Exposure** mg/m^3 (% aerosol)	**Exposure Duration**	**NOAEC LOAEC** mg/m^3	**Result**	**Reference**
Gevo (bio) 10263	0, 200, 700, 2000 (0, 0.8, 0.5, 2.0)	6 hr/dy, 5 dy/wk, 10 dy	2000 no LOAEC	No evidence of bone marrow toxicity No increase in micronuclei Non-clastogenic	Sterner *et al.* (2015a)
SB-8 8452	0, 200, 700, 2000 (0, 0, 0.04, 0.84)				Sterner *et al.* (2015b)
HEFA-C 6152	0, 200, 700, 2000 (0, 0, 0.7, 12)				Wong *et al.* (2013)
FT 5109	0, 200, 700, 2000 (0, 0.6, 12, 33)				Mattie *et al.* (2011a)

Bacterial Reverse Mutation Assay (Ames Test)

FUEL POSF	**Strains**	**Result**	**Reference**
Gevo (non-bio) 7699	*Salmonella typhimurium* TA-98, TA-100, TA-1535, TA-1537 *Escherichia coli* WP2	No evidence of mutagenicity with and without metabolic activation	Mumy *et al.* (2016)
Gevo (bio) 10263			
SB-8 8452			
SB ATJ SKA (old) 10234			Riccio *et al.* (2010)
HEFA-C 6183			Mattie *et al.* (2013)
HEFA-F 5480			Riccio *et al.* (2010)
HEFA-T 6346			Mattie *et al.* (2013)
FT 5109			Riccio *et al.* (2010)
FT 5109	*Salmonella typhimurium* TA-98, TA-100, TA-102, TA-1535, TA-1537 *Escherichia coli* WP2	No evidence of mutagenicity with and without metabolic activation	Mattie *et al.* (2011b)

LIST OF ACRONYMS

α_{2u}-globulin	alpha-2-urinary globulin
AFB	Air Force Base
ATJ	alcohol to jet
AVMA	American Veterinary Medical Association
bio	biologically-derived; renewable
CPG	chronic progressive glomerulonephropathy
CWP	Coalition Warfare Program
DoD	Department of Defense
DTIC	Defense Technical Information Center
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FOB	functional observational battery
FT	Fischer-Tropsch
FTIR	Fourier transform infrared
GC	gas chromatography
HEFA	hydroprocessed esters and fatty acids
HEFA-F	HEFA-animal mixed fats and oils
HEFA-C	HEFA-camelina
HEFA-T	HEFA-tallow (rendered beef fat)
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
JP-8	jet propulsion fuel-8
NAMRU-D	Naval Medical Research Unit – Dayton
non-bio	not biologically-derived; from petroleum sources; non-renewable
OECD	Organisation for Economic Cooperation and Development
OEL	occupational exposure level
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OSD	Office of the Secretary of Defense
PDII	primary dermal irritation index
SB	Swedish Biofuel
SD	standard deviation
SKA	synthetic kerosene with aromatics
SPK	synthetic paraffinic kerosene
WBC	white blood cell